A Backdoor to the Nucleus that Runs in the Family?

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Yoshimura and colleagues show that HEAT proteins that are involved in diverse cellular functions may facilitate their own translocation through the nuclear pore complex, owing to their structural similarity to nuclear transport receptors of the karyopherin β family.

Molecular trafficking between the nucleus and the cytoplasm flows through nuclear pore complexes (NPCs). Molecules and macromolecules roughly smaller than 40 kDa can diffuse freely through NPCs, while bulkier macromolecules must go through a well-regulated process of facilitated diffusion in which they bind to soluble transport receptors of either the karyopherin α (Kap-α) or β (Kap-β) families. Kap-αs are adaptor proteins that do not interact directly with the pore, whereas Kap-βs enable fast cargo translocation by interacting directly with disordered phenylalanine-glycine (FG) repeat domains that emanate from NPCs. At least 19 Kap-β isoforms have been identified in human and 14 in yeast (Chook and Süel, 2011).

Kap-αs and Kap-βs consist of multiple tandem repeats of the Armadillo (ARM) and closely related HEAT motif families (Andrade et al., 2001), respectively. ARM and HEAT repeats form a vast and functionally diverse group of proteins and mediate a disproportionally large number of protein interactions in the cell. It has been estimated that 1 in 500 eukaryotic proteins contains these repeats (Andrade et al., 2001). The repeats follow a loose sequence consensus, increasing the challenge in identifying members of these families on the one hand, and enabling vast functional divergence on the other.

Defying John Kendrew's famous complaint regarding "the total lack in the kind of regularities which one instinctively anticipates" in protein folds, ARM and HEAT repeats consist of a sequence of helical repeats that are stacked on top of one another with a minor clockwise twist to form a hollow super-helical structure. The resulting solenoid is highly flexible, possibly forming a soft elastic spring with fast relaxation times on the order of nanoseconds, similar to the one found in ankyrin repeats (Grinthal et al., 2010; Kappel et al., 2010; Lee et al., 2006). Multiple protein binding sites are often located on grooves along both the inner concave surface and the outer convex surface of most ARM/HEAT domains. Indeed, Kap-βs have multiple binding sites for different binding partners such as cargo molecules, the Ras-like GTPase Ran, and, importantly, FG repeat domains of nucleoporins (Figure 1A). The last interaction is critical for rapid translocation of the rather large Kap molecules and their cargo.

Certain ARM repeat proteins such as β-catenin were previously reported to cross the pore without interacting with Kaps (Fagotto et al., 1998). It was also shown that hydrophobic surface regions may increase the passive transport rate of proteins (Naim et al., 2009) and that nucleoporins Nup188 and Nup192, HEAT/ARM proteins that form a part of the NPC scaffold, could diffuse through the pore independently of Kaps (Andersen et al., 2013) but are outcompeted by high concentrations of importin-β.

In this issue of *Structure*, Yoshimura et al. (2014) report that numerous HEAT repeats that are otherwise too large to cross the NPC can interact with FG repeats and cross the pore independently of Kaps (Figure 1B). Moreover, they can interact with other proteins to mediate transport on their own. This raises the tempting possibility that HEAT proteins can bypass the classic route of transport, making use of their structure similarity to Kap-β and adapting their HEAT repeats to interact with the NPC.

Yoshimura et al. (2014) examine the role of flexibility in both importin-β and non-kap HEAT proteins and demonstrate that impaired flexibility prevents efficient nucleocytoplasmic translocation in either class. This is expected of Kap-β proteins, which are known to assume different conformations when they interact with different partners, possibly playing a role in allosteric release of cargo when exiting the pore (Conti et al., 2006). However,

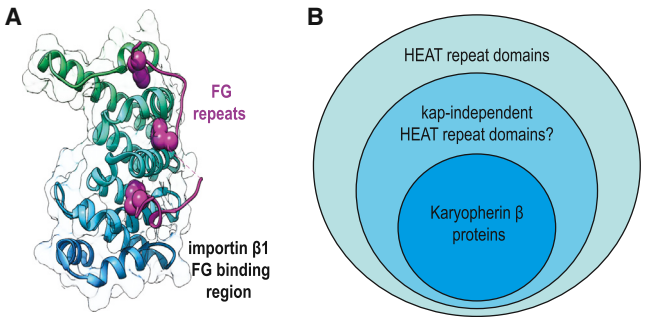

Figure 1. Putative Interactions of Non-karyopherin HEAT Proteins with NPCs
(A) The interaction between importin β1 (yeast kap95) and FG repeat domains is essential for facilitated diffusion through the NPC. Phenylalanine residues are buried in a shallow groove formed between helices of consecutive HEAT repeats.
(B) Despite their low sequence similarity, kap-β proteins are all members of the vast family of HEAT repeat domains. Yoshimura et al. (2014) provide evidence that other HEAT repeat domains, such as protein phosphatase 2A (PP2A) subunit A and CAND1, may facilitate their own transport and that of their binding partners by interacting with FG repeats.

it is intriguing that non-kap HEAT proteins also utilize flexibility in order to translocate efficiently through the pore.

In order to establish that HEAT proteins indeed act as transport factors in vivo, some important open questions need to be addressed in future studies. In the current study, translocation assays were conducted in both digitonin-permeabilized cells and live cells using fluorescence recovery after photobleaching analysis with somewhat different results in each assay. This is not surprising, considering the complexity of the protein interaction network in living cells and the likely fierce competition for FG binding sites within the cellular milieu (Jovanovic-Talisman et al., 2009; Andersen et al., 2013). For instance, the authors showed that protein phosphatase 2A (PP2A) subunit A mediates the transport of the entire trimeric PP2A complex in vitro and possibly in vivo. PP2A was previously shown to interact with two members of the Kap-β family (Lubert and Sarge, 2003), and Yoshimura et al. (2014) knocked down one of them in order to control for Kap-mediated import. However, considering the large number of different Kap-β proteins within mammalian cells, some of which are essential, it is not simple to fully establish that in vivo translocation is completely independent of Kaps. Another possibility is that by interacting with the pore, HEAT proteins like PP2A may support importins in transporting large complexes, as may be evidenced by the gradual decrease in transport rates upon knockdown of importin 7 reported in this study. This would also prevent the large cellular concentrations of importin β from outcompeting PP2A.

If HEAT proteins indeed use their kinship to nuclear transport receptors to translocate through nuclear pores, the next step would be to identify those repeats within HEAT proteins that mediate interactions with the nuclear pore and characterize the evolutionary constraints for adapting existing HEAT repeat domains for gain of access to the nucleus. We could even speculate that the first HEAT repeat domains mediated their own translocation before some of them coevolved with cargo proteins to become specialized nuclear transport receptors of the Kap family.

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New Binding Face of C-type Lectin-like Domains

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C-type lectin-like receptor 2 (CLEC-2) is a member of the C-type lectin (like) receptor (CLR) family that uses a Ca^{2+} binding domain to bind specific glycans. However, in this issue of *Structure*, Nagae and colleagues report on how the structures of CLEC-2 in complex with a glycopeptide podoplanin and a snake venom protein, rhodocytin, show a different mode of binding.

Cell surface receptors play a critical role in mediating cell-cell interactions and regulating numerous physiological events. They are also targeted by microbes that hijack them during the infection process. In turn, the host cells use their cell surface receptors as the front line defense to recognize and eliminate infectious microorganisms. Immune cell surface receptors are largely comprised of immunoglobulin (Ig) and C-type lectin (like) receptor (CLR) families (Kuroki et al.,